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Triterpenoids from the leaves of *Psidium guajava*

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Abstract

Two triterpenoids, 20β-acetoxy-2α,3β-dihydroxyurs-12-en-28-oic acid (guavanoic acid, **3**), and 2α,3β-dihydroxy-24-*p*-*z*-coumaroyloxyurs-12-en-28-oic acid (guavacoumaric acid, **7**), along with six known compounds 2α-hydroxyursolic acid (**1**), jacoumaric acid (**2**), isoneriucoumaric acid (**4**), asiatic acid (**5**), ilelatifol D (**6**) and β-sitosterol-3-*O*-β-D-glucopyranoside (**8**), have been isolated from the leaves of *Psidium guajava*. Their structures were determined through spectroscopic methods. Compound **5** showed dose-dependent (10–500 μg/ml) spasmolytic activity in spontaneously contracting isolated rabbit jejunum preparations.

Keywords: *Psidium guajava*; Triterpenoids; Guavanoic acid; Guavacoumaric acid; Spasmolytic activity

1. Introduction

Psidium guajava commonly known as guava belonging to the family Myrtaceae is a native of tropical America and has long been naturalized in southeast Asia. Different parts of the plant are used in the indigenous system of medicine for the treatment of various human ailments such as wounds, ulcers, bowels and cholera. The young leaves are used as a tonic in diseases of digestive function. The decoction of young leaves and shoots is prescribed as a febrifuge and spasmolytic. The bark is valued as an astringent and as an antidiarrhoeatic in children. The flowers are said to cool the body and are used for the treatment of bronchitis and eye sores. The fruit is a tonic and laxative and is good in bleeding gums (Krishnamurthi, 1969; Perry, 1980; Dymock et al., 1972). Phytochemical studies undertaken by different groups of workers on different parts of the plant have resulted in the isolation and identification of various terpenoids, flavonoids and tannins (Meckes et al., 1996; Lozoya et al., 1994; Seshadri and Vasishta, 1965; Tanaka et al., 1992). However, among the triterpenoids, only two pentacyclic triterpenoids of the ursane series, namely ursolic acid (Arthur and Hui, 1954) and 2α-hydroxyursolic acid (Osman et al., 1974) and three of the oleanane series, oleanolic acid (Arthur and Hui, 1954), maslinic acid (Arthur and Hui, 1954; Osman et al., 1974) and arjunolic acid (Sasaki et al., 1966) were reported earlier.

In view of the attributed medicinal properties, studies were undertaken on fresh and uncrushed leaves of the plant, which resulted in the isolation and structure elucidation of two new triterpenoids: namely, guavanoic acid (**3**) and guavacoumaric acid (**7**) along with six known compounds 2α-hydroxyursolic acid (**1**) (Yamagishi et al., 1988; Kitajima and Tanaka, 1993), jacoumaric acid (**2**) (Ogura et al., 1977; Numata et al., 1989), isoneriucoumaric acid (**4**) (Siddiqui et al., 1987), asiatic acid (**5**) (Furuya et al., 1987), ilelatifol D (**6**) (Nishimura et al., 2000) and β-sitosterol-3-*O*-β-D-glucopyranoside (**8**) (Backhouse et al., 1997). Compounds **3** and**7** have been characterized as 20β-acetoxy-2α,3β-dihydroxyurs-12-en-28-oic acid and 2α,3β-dihydroxy-24-*p*-*z*-coumaroyloxyurs-12-en-28-oic acid, respectively, based on spectral evidences. This is the first report of the isolation of compounds **2**, **4**, **5** and **6** from the genus *Psidium*.

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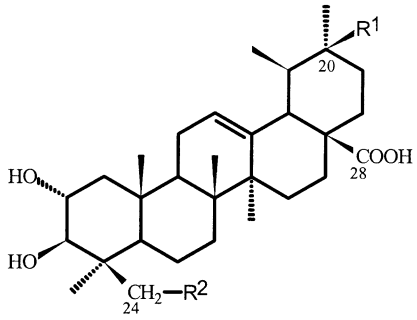
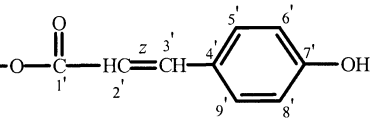


(3) R^1 = OAc, R^2 = H

(7) R^1 = H, R^2 =



Compound **5** exhibits dose-dependent (10–500 μg/ml) spasmolytic activity in spontaneous contracting isolated rabbit jejunum preparations.

2. Results and discussion

Compound **3** did not show the molecular ion peak in the EI and HREIMS and the molecular formula was established as $C_{32}H_{50}O_6$ by CIMS measurement. The ^{1}H NMR (Experimental Section) and ^{13}C NMR data (Table 1) are comparable with the reported data of **1** (Yamagishi et al., 1988; Kitajima and Tanaka, 1993) with an additional acetoxy group [δ_H 1.87 (3H, *s*); δ_C 22.1 (CH_3, DEPT); δ_C 170.1 (BB)] which could be placed at C-20 as its ^{1}H NMR spectrum showed only one methyl doublet (δ 0.70, J = 6.4 Hz) instead of two doublets in **1** and a one-proton doublet due to H-18 (Kojima and Ogura, 1986) at δ 2.03 (J = 11.8 Hz). The ^{13}C NMR data of ring *E* confirmed these assignments. The β-configuration of the acetoxy group was confirmed by NOESY interaction of OAc with H-18 and Me-29. In light of the above observations, the structure of **3** was elucidated as 20β-acetoxy-2α,3β-dihydroxyurs-12-en-28-oic acid.

Table 1
^{13}C NMR spectral data for compounds **3**[a] and **7**[b]

Carbon	Compound		Carbon	Compound	
	3	**7**		**3**	**7**
1	47.0	46.6	22	38.1	36.8
2	69.1	68.7	23	28.8	28.8
3	83.5	83.6	24	16.8	65.9
4	39.1	39.6	25	17.3	16.9
5	56.0	55.7	26	17.6	16.6
6	18.6	18.5	27	24.1	21.3
7	33.4	32.7	28	179.9	180.3
8	39.9	39.9	29	17.3	16.9
9	47.9	47.7	30	21.2	21.1
10	38.6	38.5	1′	–	168.1
11	23.9	23.4	2′	–	116.3
12	126.1	125.8	3′	–	144.7
13	139.6	139.0	4′	–	127.9
14	42.4	42.6	5′	–	133.4
15	29.1	28.2	6′	–	116.2
16	24.6	23.9	7′	–	160.2
17	48.1	48.4	8′	–	116.2
18	52.8	53.1	9′	–	133.4
19	39.1	39.2	$O\underline{C}OCH_3$	171.1	–
20	90.6	38.9	$OCO\underline{C}H_3$	22.7	–
21	31.9	30.7			

[a] Measured in $CDCl_3$–CD_3OD (1:1).
[b] Measured in CD_3OD.

2 (Haberlein and Tschiersch, 1994) values (Table 1). In view of above observations the structure of **7** was assigned as 2α,3β-dihydroxy-24-*p*-*Z*-coumaroyloxyurs-12-en-28-oic acid.

Compound **7** did not show the molecular ion peak in the EI and HREIMS. The molecular formula $C_{39}H_{54}O_7$ was derived through exact measurements of various mass fragment ions and ^{13}C NMR spectral data (BB and DEPT). Compound **7** showed IR absorptions at 3510–2620 (br, OH, COOH), 1735, 1690 (acid and ester carbonyls) and 1580–1370 (four peaks, aromatic ring) cm^{-1} while its UV spectrum showed maxima at 202, 225, 300 (sh) and 312 nm. The ^{1}H NMR and ^{13}C NMR data (Table 1) are comparable with the reported data of obtusinin (Siddiqui et al., 1990) except the two sets of doublets at δ_H 6.87 (1H, J = 12.7 Hz, H-3′) and δ_H 5.84 (1H, J = 12.7 Hz, H-2′) and δ_H 7.62 (2H, J = 8.7 Hz, H-5′ and H-9′) and δ_H 6.72 (2H, J = 8.7 Hz, H-6′ and H-8′). The chemical shifts and coupling constants of these doublets indicated the presence of *p*-*Z*-coumaroyl moiety at C-24 instead of corresponding *E* isomer which was also supported by ^{13}C NMR spectral

The structures of known compounds (**1**, **2**, **4**, **5**, **6** and **8**) were identified through comparison of their spectral (^{1}H NMR and EIMS) and physical data with those reported in literature.

The ethanol extract of the *Psidium guajava* leaves along with its pure compounds **1, 5** and **8** were tested for possible spasmolytic activity via their effects on the spontaneous movements of the isolated rabbit jejunum preparations. The plant extract caused dose-dependent

Table 2
Spasmolytic activity of compound **5** in spontaneously contracting isolated rabbit jejunum

Dose (μg/ml)	Effect (% relaxation)
10	13.1±4.9
30	40.4±0.5
100	53.8±4.5
300	71.1±3.3
500	84.5±3.0

Values shown represent means±S.E.M. of 4 determinations, with EC_{50} = 80.0±7.8 μg/ml.

(0.1–3.0 mg/ml) relaxation of spontaneous contractions with median effective dose (EC_{50}) of 0.56 ± 0.04 mg/ml (mean ± S.E.M.; $n = 4$), which confirms the spasmolytic effect of *Psidium guajava* leaves reported in earlier studies (Lozoya et al., 1994).

Compounds **1** and **8** were found inactive up to the dose of 300 μg/ml, whereas compound **5** showed dose-dependent (10–500 μg/ml) relaxation of spontaneous contractions with EC_{50} value of 80 ± 7.8 μg/ml (Table 2). Our previous study (Begum et al., 2000) showed that the spasmolytic activity of the active constituents was mediated through blockade of calcium channels. Similarly, a flavonoid (quercetin) from the leaves of this plant has also been shown to mediate spasmolytic action through calcium antagonist activity (Morales et al., 1994). Experiments were performed to see whether the spasmolytic activity obtained in the present study is through similar mechanism. However, compound **5** did not show any relaxant activity when tested against K^+ (50 mM) mediated depolarization of the tissue (data not shown), indicating that it is devoid of such mechanism in its spasmolytic activity (Farre et al., 1991). The activity of the rest of the compounds could not be determined in present studies due to their limited quantities.

The leaves of this plant have been used traditionally in the hyper motility of gut as spasmolytic in diarrhoea (Lozoya et al., 1994). It is likely that the presence of compound **5** along with some previously reported compounds including quercetin glycosides in the leaves of this plant (Lutterodt, 1989; Morales et al., 1994; Lozoya et al., 1994) are responsible for the spasmolytic properties reported in the folklore (Brunton, 1996) and multiple compounds acting on different sites may make the plant leaves more effective in the treatment of diarrhoea.

3. Experimental

3.1. General

Melting points were determined using a Gallenkamp melting point apparatus and are uncorrected. Ultraviolet spectra were measured on a Hitachi U-3200 spectrophotometer. Infrared spectra were recorded on a JASCO A-302 spectrophotometer. The ^1H, and ^{13}C NMR spectra were recorded on a Bruker Aspect AM 400 spectrometer operating at 400 MHz (^1H) and 100 MHz (^{13}C). Mass spectra were run on a Jeol JMS-HX110 (high-resolution, e.i. probe, 70 eV) and a Varian MAT 311 A (low-resolution, e.i. probe, 70 eV) instrument. Thin layer chromatography (TLC) was performed on Merck precoated silica gel 60 F_{254} plates. For vacuum liquid chromatography (VLC) silica gel (Merck 60 F_{254}) and for flash column chromatography (FCC, Model Eyela EF 10) silica gel (Merck 9385) were used. Petrol refers to a mixture of alkanes which boils at 60–80°C.

3.2. Plant material

The leaves of the plant were collected from the Karachi region. The plant was identified by Dr. Mohammad Qaiser, Department of Botany, University of Karachi, Karachi, Pakistan and a voucher specimen [KUH-GH No. 53976] has been deposited in the Herbarium.

3.3. Extraction and isolation

Fresh, undried, and uncrushed leaves (20 kg) of *Psidium guajava* were extracted (3 times) with ethanol at room temperature. The concentrated syrupy residue obtained after reduction in vacuum was partitioned between EtOAc and H_2O. The EtOAc layer was washed, dried (Na_2SO_4), treated with charcoal and filtered. The charcoal bed was further washed with MeOH–C_6H_6 (1:1). The residue left on removal of the solvent from the EtOAc filtrate and MeOH–C_6H_6 washings were combined on the basis of TLC analyses and freed of the solvent under reduced pressure. The residue obtained was divided into petrol-soluble and petrol-insoluble fractions. The residue left on solvent removal from the petrol soluble fraction was partitioned between 90% aqueous MeOH and petrol. The residue (45 g), obtained on usual work-up of 90% aqueous MeOH phase, was subjected to VLC (petrol, petrol–EtOAc in increasing order of polarity). Various fractions obtained on elution were combined on the basis of TLC to ultimately give 11 fractions (G-I to G-XI). The fraction G-IX (5.0 g, petrol–EtOAc, 8:2 eluate) was subjected to VLC ($CHCl_3$, $CHCl_3$–MeOH, in increasing order of polarity) and nine fractions (G-IX-1 to G-IX-9) were obtained. The residue obtained from G-IX-3 ($CHCl_3$–MeOH, 9.8:0.2 eluate) and G-IX-4 ($CHCl_3$–MeOH, 9.7:0.3 eluate) afforded **1** (237 mg) as a colorless crystalline solid when left in $CHCl_3$–MeOH (1:1) at room temperature overnight. The residue (622.3 mg) obtained on removal of the solvent from the mother liquor of G-IX-3 was further subjected to FCC ($CHCl_3$, $CHCl_3$–MeOH, in increasing order of polarity) to give 8 fractions. Fraction 3 ($CHCl_3$–MeOH, 9.9:0.1 eluate) (37.9 mg) showed two separate spots on TLC plates ($CHCl_3$–MeOH, 9.5:0.5) affording compound **2** (18.0 mg) and compound **1** (16.5 mg) in order of polarity. Fraction 5 ($CHCl_3$–MeOH, 9.8:0.2 eluate) showed one main spot on TLC which was purified on TLC cards (E. Merck Kieselgel Si-F_{254} precoated aluminum cards, $CHCl_3$–MeOH, 9.5:0.5) to give **3** (6.8 mg). The residue (437.3 mg), obtained on removal of the solvent from the mother liquor of G-IX-4, was further subjected to FCC ($CHCl_3$, $CHCl_3$–MeOH, in increasing order of polarity) to give 14 fractions. Fraction 4 ($CHCl_3$–MeOH, 9.9:0.1 eluate) and fraction 10 ($CHCl_3$–MeOH, 9.8:0.2 eluate) gave **4** (9.0 mg) and **5** (246.8 mg) respectively. Fraction G-IX-5 ($CHCl_3$–MeOH, 9.5:0.5 eluate) (1.00 g) was

subjected to FCC ($CHCl_3$, $CHCl_3$–MeOH, in increasing order of polarity) which afforded 13 fractions. Fraction 3 ($CHCl_3$–MeOH, 9.8:0.2 eluate) gave **6** (10.0 mg) and fraction 5 ($CHCl_3$–MeOH, 9.8:0.2 eluate) afforded **7** (7.0 mg). The residue obtained from fraction 9 was kept overnight at room temperature in $CHCl_3$–MeOH (1:1) affording compound **8** (61.9 mg) as a colorless crystalline solid. The residue (255.9 mg), obtained on evaporating the solvent from the mother liquor, was subjected to FCC ($CHCl_3$, $CHCl_3$–MeOH, in increasing order of polarity) affording 8 fractions. Fraction 4 ($CHCl_3$–MeOH, 9.9:0.1 eluate) gave **5** (136.5 mg).

3.4. Guavanoic acid (*3*)

Colourless needles ($CHCl_3$–MeOH 1:1); mp 221–222 °C; UV λ_{max}^{MeOH} nm: 205; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 3430–2600 *br*, 1735, 1700, 1610; ^1H NMR spectral data (CD_3OD-$CDCl_3$ 1:1): δ 5.08 (1 H, *t*, $J = 3.2$ Hz, H-12), 3.50 (1H, *ddd*, $J = 11.3$, 10.0, 4.5 Hz, H-2β), 2.78 (1H, *d*, $J = 10.0$ Hz, H-3α), 2.03 (1H, *d*, $J = 11.8$ Hz, H-18), 1.87 (3H, *s*, 20-OAc), 0.93 (3H, *s*), 0.86 (3H, *s*), 0.83 (3H, *s*), 0.79 (3H, *s*), 0.70 (3H, *d*, $J = 6.4$ Hz, H-29), 0.66 (3H, *s*), 0.65 (3H,s); ^{13}C NMR spectral data are shown in Table 1; positive CIMS *m*/*z*: 531 $[M + 1]^+$ (4).

3.5. Guavacoumaric acid (*7*)

Colourless needles ($CHCl_3$–MeOH 1:1); mp 188–190 °C; UV λ_{max}^{MeOH} nm: 202, 225, 300(*sh*) and 312; IR ν_{max}^{KBr} cm^{-1}: 3510–2620, 1735, 1690, 1580–1370; ^1H NMR spectral data (CD_3OD): δ 7.62 (2H, *d*, $J = 8.7$ Hz, H-5′ and H-9′), 6.87 (1H, *d*, $J = 12.7$ Hz, H-3′), 6.72 (2H, *d*, $J = 8.7$ Hz, H-6′ and H-8′), 5.84 (1H, *d*, $J = 12.7$ Hz, H-2′), 5.23 (1H, *t*, $J = 3.4$ Hz, H-12), 4.63 (1H, *d*, $J = 10.0$ Hz, H-24a), 4.58 (1H, *d*, $J = 10.0$ Hz, H-24b), 3.78 (1H, *ddd*, $J = 10.5$, 9.5, 4.5 Hz, H-2β), 2.90 (1H, *d*, $J = 9.5$ Hz, H-3α), 1.14 (3H, *s*), 1.04 (3H, *s*), 1.01 (3H, *s*), 0.95 (3H, *d*, $J = 6.5$ Hz), 0.88 (3H, *d*, $J = 6.6$ Hz), 0.82 (3H, *s*); ^{13}C NMR spectral data are shown in Table 1; EIMS *m*/*z*: 470 (20), 452 (9), 424 (10), 248 (100), 203 (62), 185 (16), 164 (45), 147 (16), 133 (52); HREIMS *m*/*z*: 470.3392, [M- *p*-coumaric acid]$^+$, $C_{30}H_{46}O_4$ requires 470.3395.

3.6. Spasmolytic activity

Spasmolytic activity of the test compounds was studied by using isolated rabbit jejunum preparations, as described previously (Gilani et al., 1994). Rabbits (1.5–2.0 kg) of local Desi breed and either sex, housed at the Animal House of The Aga Khan University, Karachi, were used for this study. Segments of 2 cm length were suspended in Tyrode's solution aerated with a mixture of 95% oxygen and 5% carbon dioxide, maintained at 37 °C. The composition of the Tyrode's solution was: KCl 50, NaCl 91.04, $MgCl_2$ 1.05, $NaHCO_3$ 11.87, NaH_2PO_4 0.41, $CaCl_2$ 1.8 and glucose 5.55 mM. Intestinal responses were recorded isotonically using BioScience transducers and an oscillograph. Each tissue was allowed to equilibrate for at least 30 min before the addition of any metabolite.

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